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FIELD EVALUATION OF 'CERCOSPORA RODMANII' AS A BIOLOGICAL CONTR--ETC(U)
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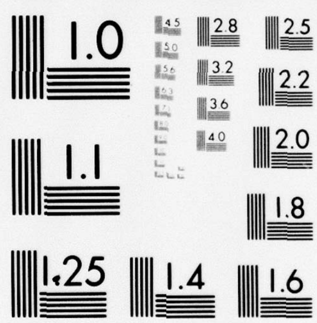

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MISCELLANEOUS PAPER A-79-6

FIELD EVALUATION OF *CERCOSPORA RODMANII* AS A BIOLOGICAL CONTROL OF WATERHYACINTH

Inoculum Rate Studies

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October 1979

Final Report

Prepared for Office, Chief of Engineers, U. S. Army
Washington, D. C. 20314

Under Contract No. DACW39-76-C-0097

Monitored by Environmental Laboratory
U. S. Army Engineer Waterways Experiment Station
P. O. Box 631, Vicksburg, Miss. 39180

Unclassified
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Miscellaneous Paper A-79-6	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) 6 FIELD EVALUATION OF *CERCOSPORA RODMANII* AS A BIOLOGICAL CONTROL OF WATERHYACINTH; Inoculum Rate Studies.		5. TYPE OF REPORT & PERIOD COVERED 9 Final report.
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) 10 K. E. Conway, R. E. Cullen, T. E. Freeman J. A. Cornell		8. CONTRACT OR GRANT NUMBER(s) Contract No. 15 DACW39-76-C-0097
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Florida Department of Plant Pathology Gainesville, Fla. 32601		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Office, Chief of Engineers, U. S. Army Washington, D. C. 20314		12. REPORT DATE 11 October 1979
		13. NUMBER OF PAGES 51
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) U. S. Army Engineer Waterways Experiment Station Environmental Laboratory P. O. Box 631, Vicksburg, Miss. 39180		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

12 56

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18 WES   19 MP-A-79-6

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Aquatic plant control	Fungi
Biological control	Fungicides
Cercospora rodmanii	Inoculation
Florida	Water hyacinths

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The fungus Cercospora rodmanii Conway has been shown to have good potential as a biological control agent for waterhyacinth in Florida. Previous work has shown what will happen if known quantities of the fungus are placed on an area of waterhyacinth. This current research addresses the study as optimal levels of inoculum necessary to begin an epidemic, various levels of inoculum on small populations of waterhyacinth, morphological changes of the infested plants, the effect of subsequent inoculations on infested plants,

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20. ABSTRACT (Continued).

and the effect of off-the-shelf fungicides on Cercospora rodmanii. It was shown that the fungus can severely affect waterhyacinth in conditions that favor a reduced growth of the plant. The Cercospora rodmanii can be controlled with available fungicides if necessary. The greatest effect of the fungus on waterhyacinth was in reduction in height of the plants. Secondary infestations can occur with the spread of the disease from inoculated plants.

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Preface

This report presents partial results of research with plant pathogens for the biological control of waterhyacinths being conducted for the Aquatic Plant Control Research Program (APCRP) by the University of Florida, Department of Plant Pathology, Gainesville, Florida, under Contract No. DACW39-76-C-0097.

The overall investigation was supported in part by the U. S. Department of Interior, Office of Water Resources and Technology, as authorized under the Water Resources Research Act as amended, the U. S. Army Corps of Engineers, and the Florida Department of Natural Resources. Funds for the Corps' part of this effort were provided by the Office, Chief of Engineers, under appropriation number 96X3122, Construction General, through the APCRP at the U. S. Army Engineer Waterways Experiment Station (WES).

The principal investigator for the contract under which this work was a part was Dr. T. E. Freeman, University of Florida. Dr. K. E. Conway directed the work reported herein. This report was written by Drs. K. E. Conway, R. E. Cullen, T. E. Freeman, and J. A. Cornell.

The authors would like to extend a special appreciation to Mr. John Thrasher and his father for the utilization of their lake as the experimentation site for the past three years. The authors would also like to extend thanks to the following people who assisted in the establishment of the test in the lake and for collection of data throughout the experiment: M. Nadeau, C. Hennen, E. Shepack, D. Reese, and K. Hencin.

The work was monitored at WES by Mr. W. N. Rushing of the Aquatic Plant Research Branch (APRB), under the general supervision of Mr. W. G. Shockley, Chief of Mobility and Environmental Systems Laboratory (MESL), and Mr. B. O. Benn, Chief of the Environmental Systems Division, and under the direct supervision of Mr. J. L. Decell, Chief of the APRB. Mr. Decell is now manager of the APCRP, which is a part of the Environmental Laboratory (EL). Dr. John Harrison is Chief of EL.

The Commanders and Directors of WES during this period were COL John L. Cannon, CE, and COL Nelson P. Conover, CE. Technical Director was Mr. F. R. Brown.

Contents

FIELD EVALUATION OF *CERCOSPORA RODMANII* AS A BIOLOGICAL CONTROL OF WATERHYACINTH

INOCULUM RATE STUDIES

Introduction

1. The fungus *Cercospora rodmanii* Conway has been shown to have good potential as a biological control for waterhyacinth in Florida (Conway 1976, Conway and Freeman 1976). In most previous research, epidemics of the disease were initiated by application of a known weight of the fungus onto an area of waterhyacinth. Therefore, this research was initiated to quantify the effect of *C. rodmanii* on limited populations of waterhyacinth. The objectives to this research were to:

a. Determine if there was an optimal inoculum concentration of the fungus to begin an epidemic.

b. Determine what effect various levels of inoculum had on limited populations of waterhyacinth over a period of time.

c. Determine what morphological changes occurred on plants infected with *C. rodmanii*.

d. Determine if a second inoculation of the fungus onto waterhyacinth populations in the fall of the year would increase disease severity.

e. Determine if the disease could be controlled on the waterhyacinth by the use of available fungicides.

Materials and Methods

2. The lake (1.6 ha) used in this study was located in Fish Prairie, near Micanopy, Florida. The experimental design of the study is illustrated in Figure 1. Thirty-five polyvinyl chloride (PVC) frames (5.08 cm in outside diameter) were constructed so that each enclosed an area of 9 m^2. A galvanized wire screen was attached to the PVC to prevent the movement of waterhyacinth out of the frames (Figure 2). A wire was strung along one side of the lake and was supported from posts that had been driven into the bottom of the lake. The frames

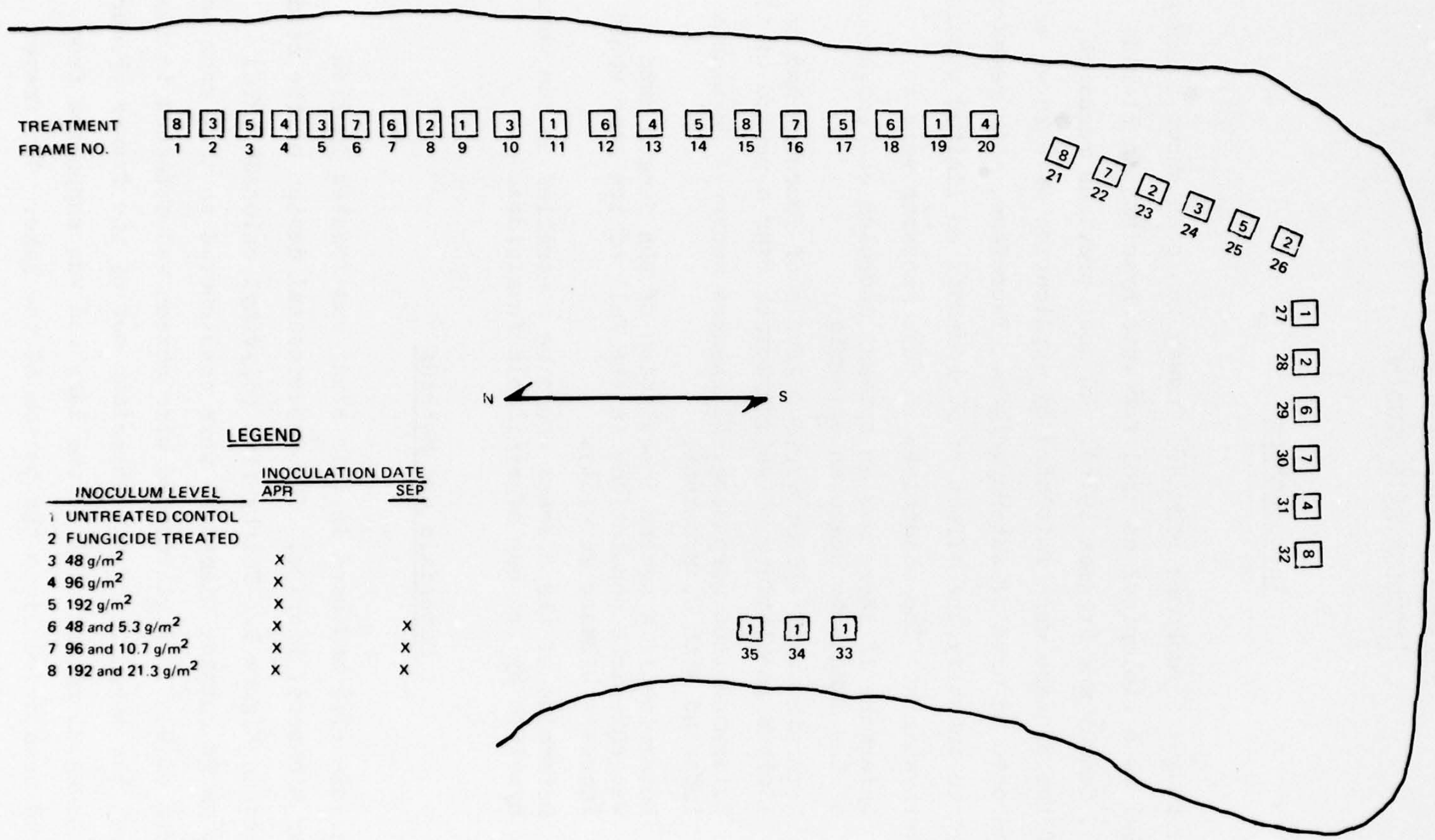


Figure 1. Experimental design in Trasher Pond

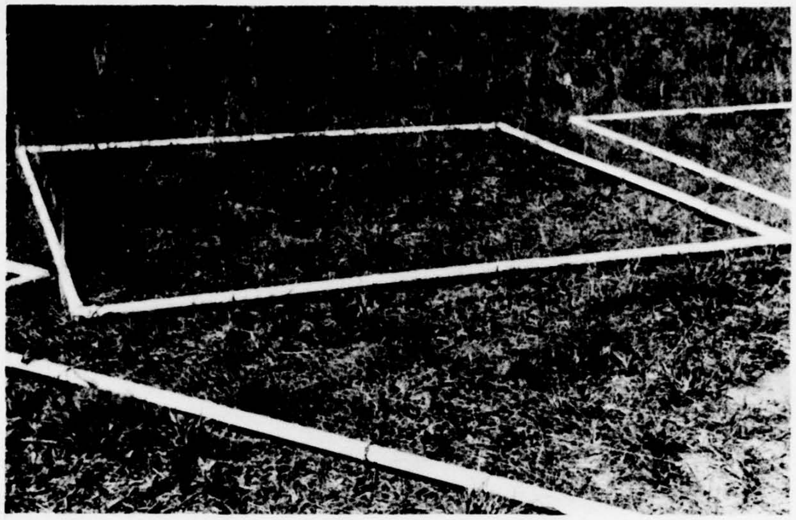


Figure 2. Structure of the PVC frames showing the wire barrier surrounding the frames

were attached to this wire and each frame was separated from the next by a distance of at least 2 m (Figure 3). The inoculum rate test consisted of a string of 32 frames. Three additional frames were anchored approximately 35 m from this test and were used as extra untreated control plots.

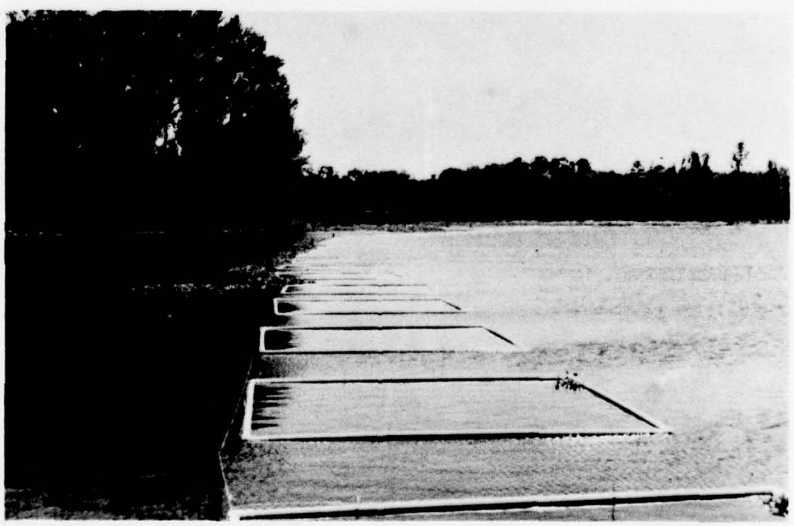


Figure 3. Placement of the PVC frames in the lake showing attachment to poles and wire

3. Three basic inoculum rates were used in this experiment based on previous studies (Conway and Freeman 1976). The inoculum used consisted of a mycelial suspension which was applied at concentrations of 48, 96, and 192 g/m^2. These basic rates were designated treatments T-3, T-4, and T-5, respectively. Initially, each inoculum rate was applied to waterhyacinth in eight of the frames. Waterhyacinth in four of the remaining frames were left as untreated controls (T-1) and waterhyacinth in the last four frames were treated with a fungicide (T-2). In the fall of the year, waterhyacinth in four frames from each basic rate received additional inocula at a rate proportional to the initial rate (5.3, 10.7, and 21.3 g/m^2), and these treatments were designated T-6, T-7, and T-8, respectively. Data for plots receiving these treatments were recorded separately from plots receiving just the basic rates even though they represented the same rate until the second inoculation. However, when the data are pooled in the Results and Discussion section, the inoculum levels will be designated T-(3-6), etc.

4. All frames were originally stocked with 100 waterhyacinth plants (Figure 4). These plants were collected from the resident plant population of the lake and were trimmed so that only two to three healthy

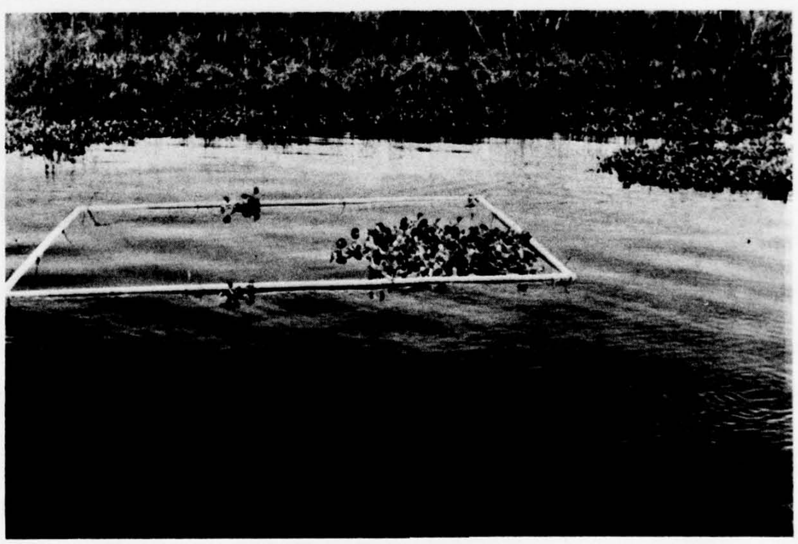


Figure 4. Initial coverage of the original stock of 100 waterhyacinth plants, 15 April 1976

leaves remained. The older, frost, or otherwise, damaged leaves were removed. Frames were stocked during the first week of March, and the plants were allowed to stabilize for approximately 1 month before they were inoculated with the fungus. The area covered by the plants in the frames at the time of inoculation was approximately 1 m^2.

5. Treatments were randomly assigned to the frames (Figure 1), although a slight bias was interjected to avoid the placement of the higher treatments (T-(5,8)) in juxtaposition with the untreated controls (T-1).

6. The fungus was grown on potato-dextrose broth with 0.5 percent yeast extract added. The mycelial mats of *C. rodmanii* were harvested at the end of 17 days and comminuted in a blender. The fungus was then applied to waterhyacinth with a portable power pump sprayer with a hollow cone nozzle which had been calibrated to deliver 1 ℓ of inoculum in a 10-sec period. Proper dilutions of inocula were prepared and the fungus was applied to the plants in the frames between 5:00 and 6:30 p.m. on 22 April 1976. The air temperature was 23°C.

7. The second inoculation of *C. rodmanii* to waterhyacinth in treatments T-6, T-7, and T-8 was applied on 30 September 1976. Application was completed between 5:30 and 6:30 p.m.; the air temperature was 23° to 24°C. Due to limited production facilities, the original rate per square metre could not be duplicated. However, the same weight of inoculum was applied per frame except that it was applied to a 9-m^2 area of plants since the frames were completely filled with waterhyacinth at that time.

8. Data were collected at approximately 3-week intervals. Data collected included: visual assessment of plots, disease damage per leaf, the number of leaves (both emergent and submergent) per plant, height of the longest leaf per plant, and the length of the longest root per plant (Table 1). Damage per leaf was based on a rating scale of 0 to 9 (Table 2) where 0 meant no apparent infection on the leaf and 9 indicated a dead, submerged leaf blade and petiole. The values between 1 and 8 corresponded to increasing coverage (damage) of the leaf blade by *C. rodmanii*. Total damage to the plant was a sum of the damage

to individual leaves. Data also indicated the number of dead leaves and the number of emergent leaves per plant. Data were recorded from subsample populations that consisted of 10 plants selected at random from each plot. For the first six sampling periods, only the original plants that had been stocked in the frames were sampled. After that time, older offshoots (plants derived from the original mother stock) were sampled due either to the death of the original plants or the difficulty in identifying them in the population of plants.

9. Two fungicides were used to control the disease on waterhyacinth: Daconil 2787 (Diamond Shamrock) and Benlate (Dupont). In order to avoid the possibility of a fungicide-resistant strain of *C. rodmanii* developing, the fungicides were at first alternated on a 10- to 14-day spray schedule. It was known from previous experiments (Conway and Freeman 1976) that the disease would spread to infect the untreated controls within a few months. Therefore, the fungicide-treated plants would act as a baseline that would indicate how plants in the lake functioned without the stress of the disease.

10. Data were analyzed by computer, and for each sampling date an analysis of variance was performed in order to test for differences among the treatments. For comparisons between pairs of treatment means, the standard t-test (Steel and Torrie 1960) was employed, and significant differences were usually recorded only at the 0.05 level. Occasionally, highly significant responses (0.01) were observed and these, along with less significant indications (0.1 or less), will be noted in Table 3 and the Results section. Regression slope analyses were performed on the first three and the last four sampling dates for the following variables: number of emergent leaves per plant, total damage per plant (emergent leaves), and total damage per emergent leaf.

Results

Number of leaves per plant (Figure 5)

11. This variable represented a count of all leaves, both emergent and submergent, on each plant sampled. At the beginning of the

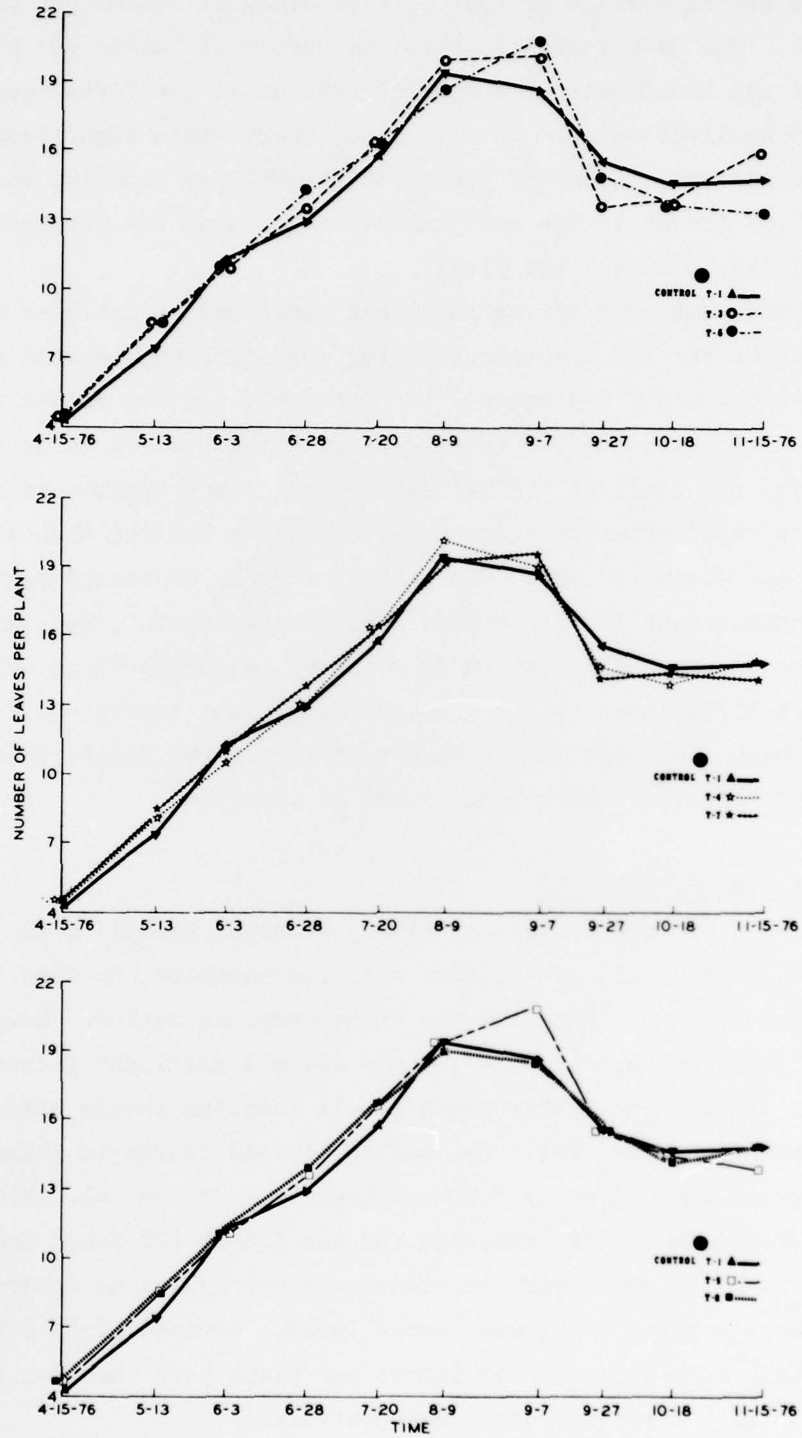


Figure 5. Number of leaves per plant versus time

test there was an average of four to five emergent leaves per plant in all frames. The data indicated that the number of leaves per plant increased in all treatments at a similar rate until the fifth sampling period (15 April-20 July). On this date, there was a significant difference between the number of leaves at the highest inoculum concentration, T-(5,8) (16.85 leaves per plant), compared to the untreated controls, T-1 (15.70 leaves per plant).

12. The number of leaves per plant continued to increase and reached maxima for all treatments during the sixth and seventh sampling dates (9 August and 7 September). On 9 August, maximum values were reached in T-1, T-4, and T-8 treatments with the highest value being 20.13 leaves per plant at the T-4 level. The other treatments reached peak values on 7 September and the T-5 treatment had the highest number of leaves per plant (20.93). The numbers rapidly decreased by the eighth sampling date (27 September) and, except for T-5, T-6, and T-7, all treatments reached minima on 18 October. Although there were no significant differences on the last sampling date, treatments T-5, T-6, and T-7 showed a decline in the number of leaves per plant, whereas the other treatments, including T-8, showed an increase.

Number of dead
leaves per plant (Figure 6)

13. When the frames were initially stocked, plants in the frames had been cleaned of all dead leaves and, consequently, no dead leaves were recorded on the plants for the first sampling period. However, by the third sampling date (3 June), there was a significant increase in the number of dead leaves per plant at all inoculum levels compared to the untreated controls, T-1. The number of dead leaves in these treatments averaged approximately 2.20 compared to 1.39 for T-1. Significant differences were also recorded for the fourth (28 June) and fifth (20 July) sampling dates for each treatment versus T-1 as determined by using Dunnett's test (Steel and Torrie 1960). Treated plots averaged approximately 1.20 and 0.9 dead leaves per plant more than T-1 for the fourth and fifth sampling dates, respectively.

14. The number of dead leaves per plant increased in all

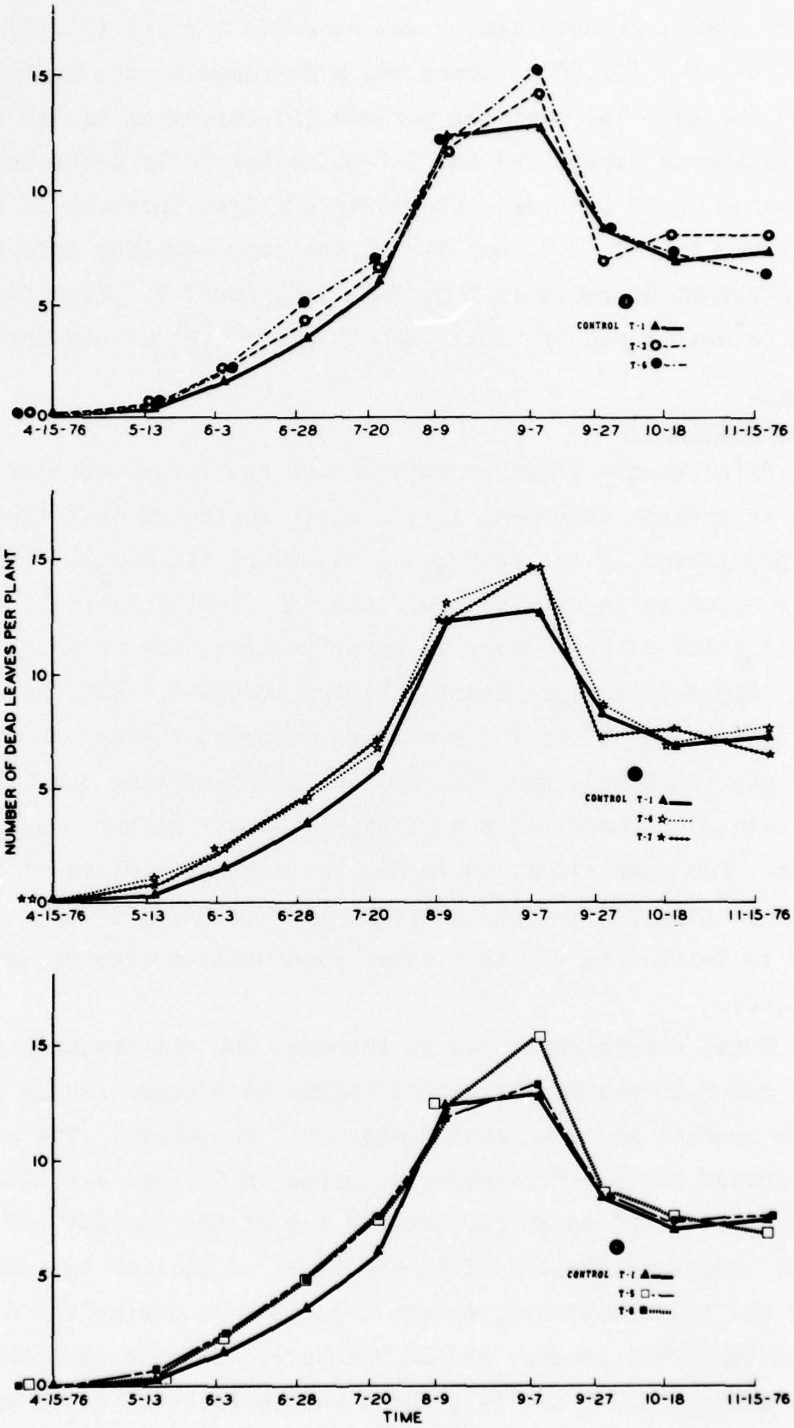


Figure 6. Number of dead leaves per plant versus time

treatments and reached maxima on the seventh sampling date (7 September). The highest number of dead leaves was recorded for T-5 (15.25) while the lowest was T-1 (12.68). There was a decrease in the number of dead leaves for the next two sampling periods (27 September and 18 October) for all treatments except T-3 and T-7 which initially decreased and then increased on 18 October. There was a slight increase in the number of dead leaves in T-1, T-4, and T-8 on the last sampling date (15 November) and a slight decrease in T-3, T-5, T-6, and T-7. Significant responses were determined by using Dunnett's test (Steel and Torrie 1960).

Total damage
per plant (Figure 7)

15. Prior to the first inoculation of the fungus onto waterhyacinth, an assessment of damage in the plots indicated that there was a resident population of the pathogen present and the highest incidence of disease occurred in T-(3,6) plots and the least in T-(4,7) plots. After application of the fungus to waterhyacinth, the disease ratings for total damage were significantly higher (0.01) for all treatments compared to T-1. These differences were recorded through the fourth sampling date (28 June), whereas, on the fifth sampling date (20 July), only the T-(5,8) treated plots were significantly higher than the T-1 treatments. For comparison, on 13 May the assessed values of damage were twice as high for T-(5,8) compared to T-1 (32.83:15.87). However, as damage to T-1 increased, this ratio eventually decreased by 28 June (66.92:57.00).

16. Total damage continued to increase for all treatments as the T-1, T-4, and T-8 treatments reached maxima on 9 August while the other treatments reached maximum total damage on 7 September. The highest damage recorded for the treatments occurred in T-5 and T-6 (148.58 and 148.57, respectively) which represented one of the highest and one of the lowest inoculum levels used in the test. A decline in total damage was noted for all treatments, except T-3 and T-7, during the next two sampling dates (27 September and 18 October). After an initial decrease in damage in T-3 and T-7, there was an increase noted by 18 October. On the last sampling date all treatments showed an increase

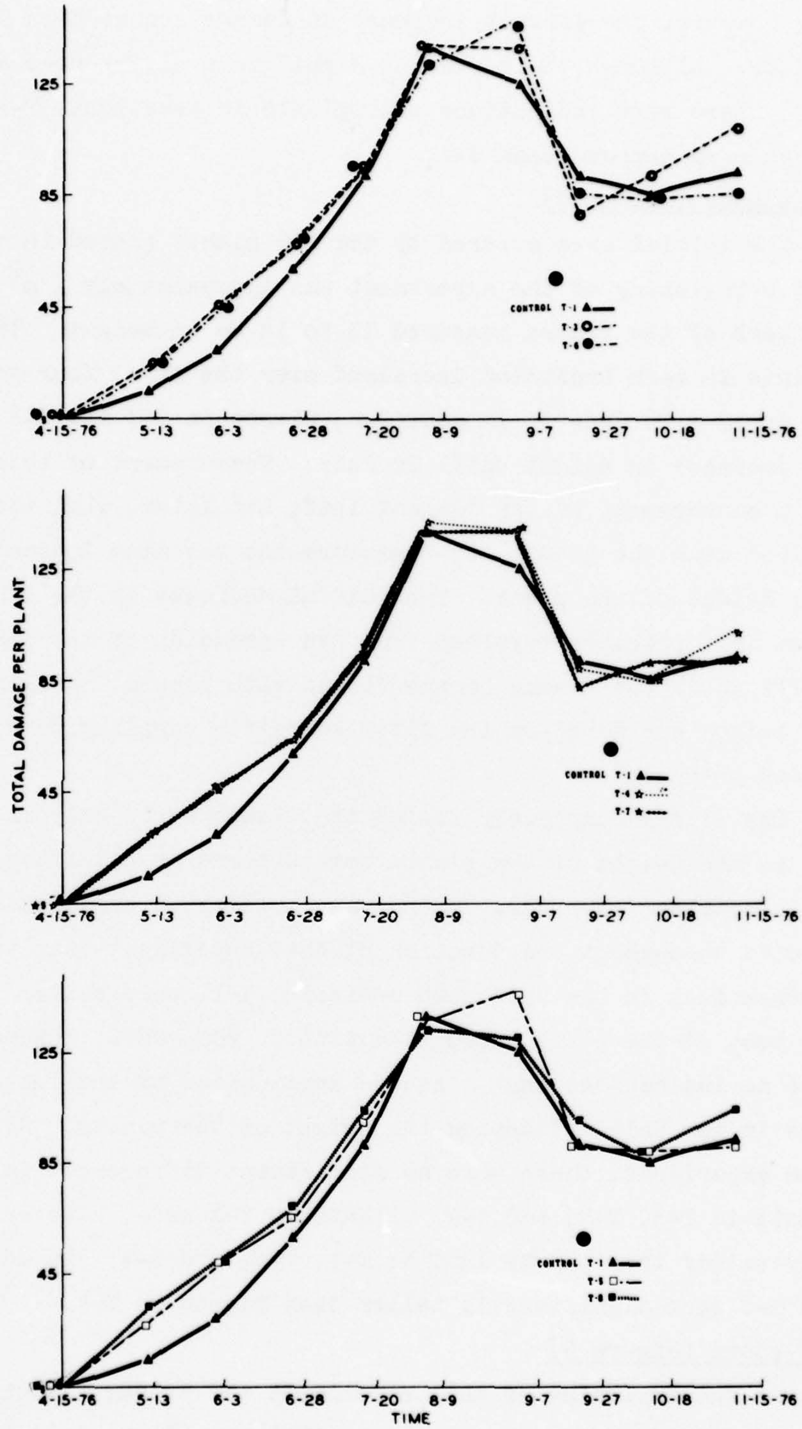


Figure 7. Total damage per plant versus time

in damage; however, the largest increase in damage occurred in T-3, T-4, and T-8 plots. Although there were no significant differences among treatments, there were indications that plants in treatments T-3, T-4, and T-8 were more damaged than T-1.

Height of plants (Figure 8)

17. The initial area covered by the 100 plants placed in each 9-m^2 frame at the beginning of the experiment was approximately 1 m^2, and plants in each of the frames measured 13 to 14 cm in height. The height of the plants in each treatment decreased over the first four sampling dates (15 April - 28 June). In addition, plants in T-7 and T-8 continued to decrease in height until 20 July. Measurement of height was initially a measurement of the longest leaf; but later, when the frames became filled with the plants, this measurement was also indicative of the actual height of the plant. The initial decrease in the length of the longest leaf probably resulted from the spreading of the plants horizontally until the frames became filled with plants. A slight increase in height was noted on the fifth and sixth sampling dates (20 July and 9 August).

18. The plants completely filled the frames by 20 July and larger increases in the height of the plants were noticed in all frames following this complete coverage. There was, however, a significant difference noted throughout the duration of this experiment that indicated that waterhyacinth in the untreated controls, T-1, were taller than the plants in most of the plots inoculated with *C. rodmanii*. However, there were no indications that a second application of the fungus to the plants in the fall influenced the height of the plants. At the end of the experiment, there were no significant differences in height among plants in T-3, T-8, and T-1. Plants in T-1 were, however, significantly taller than plants in T-4, T-5, T-6, and T-7. In addition, plants in T-8 were significantly taller than plants in T-4.

Length of roots (Figure 9)

19. At the beginning of this experiment the average length of the roots varied significantly from 11.40 cm (T-7) to 16.01 cm (T-1). However, by 13 May root lengths were similar in all plots except that there

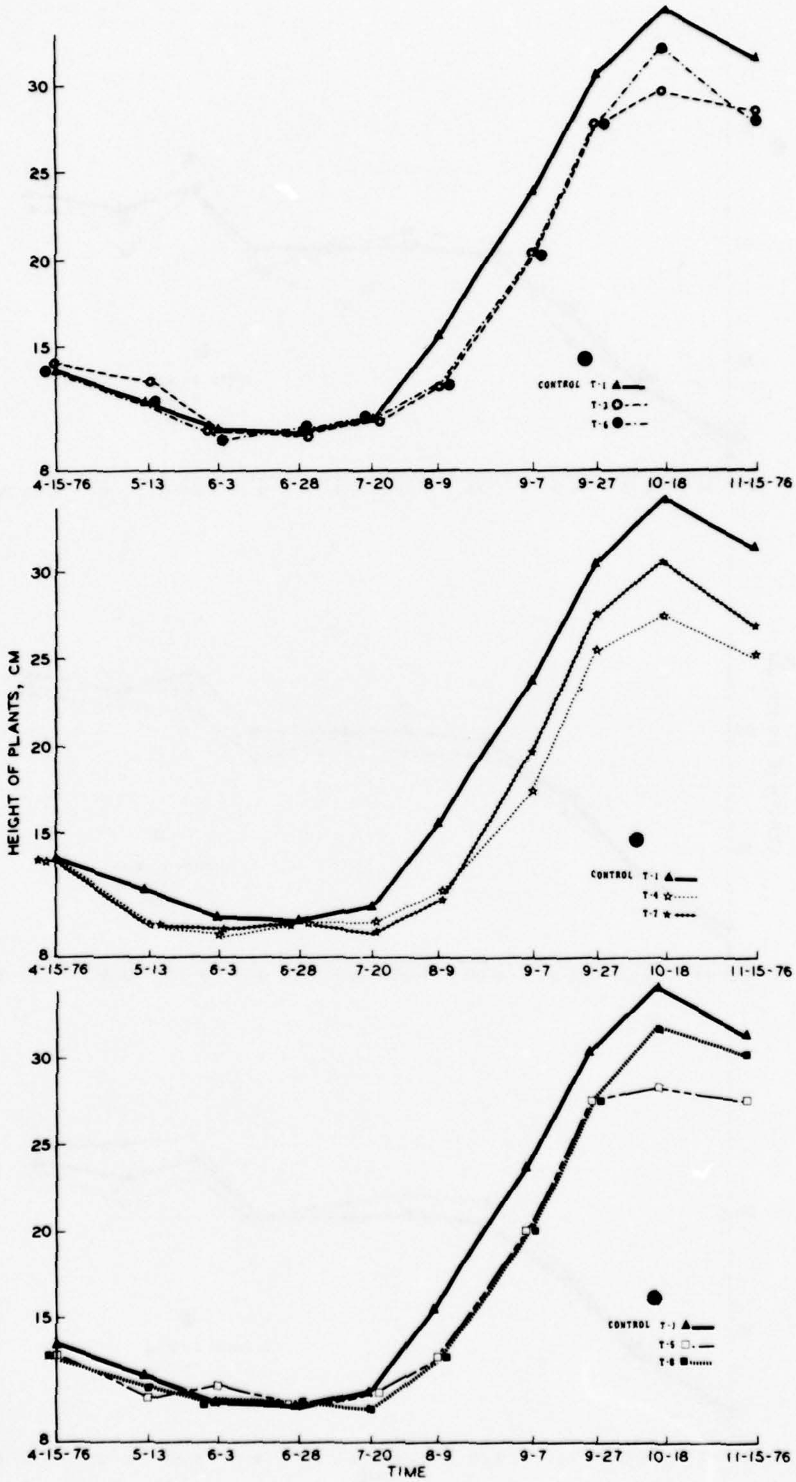


Figure 8. Height of plants versus time

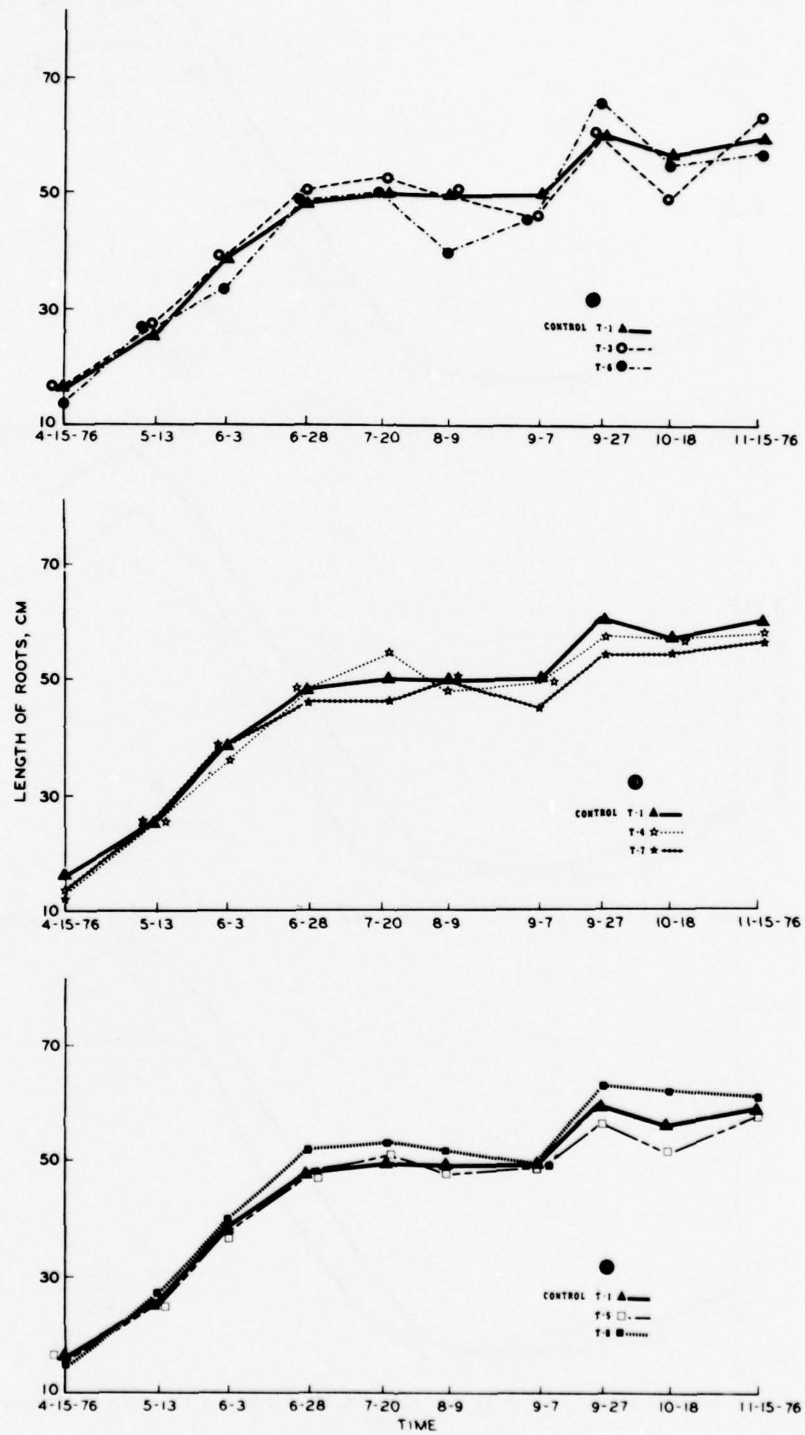


Figure 9. Length of roots versus time

were indications that roots of plants in T-3 were longer than T-5 and T-7. The length of the roots of waterhyacinth in all treatments increased with time. The initial increase in the length of the roots, which occurred during the sampling period 15 April to 28 June, corresponded to the horizontal spread of the plants in the frames. Once the plants became established, the root growth remained more or less constant until 7 September. However, plants in the T-(5,8) plots had slightly longer root systems during the month of June.

20. There was a second increase in root length from 7 September to 27 September, with the greatest increase in length recorded for the T-6 treatment; however, this was not significantly different from the other treatments. After the second application of the fungus to the plants (T-6, T-7, and T-8) on 30 September, there was a general reduction in root length in all treatments except the T-4 and T-7 inoculum levels. On 18 October there was a significant difference between the T-8 compared to the T-3, T-5, and T-7 treatments, with the T-8 being longer (62.48 cm versus 49.35, 51.84, and 53.90 cm, respectively). There were no significant differences recorded among the treatments at the end of the testing period (15 November); however, the greatest length was recorded for plants in T-3 plots which represented the greatest average increase for any treatment. Plants in T-7 had the shortest roots (55.91 cm) at that time.

Number of emergent leaves per plant (Figure 10)

21. Waterhyacinth in all plots initially averaged between 4.3 and 4.7 emergent leaves per plant. The number of emergent leaves per plant increased in all treatments for the first month after application of *C. rodmanii* to the waterhyacinth. There were indications on the second sampling date (13 May) that plants in T-1 had fewer leaves than plants in the T-(3,6) plots. However, by 3 June and 28 June, T-1 plots averaged 10.0 to 10.5 emergent leaves per plant and had significantly more emergent leaves per plant than any of the other treatments. The number of emergent leaves reached maxima in all treatments on 20 July with T-1 plants averaging 10.5 leaves compared to approximately 10 leaves per

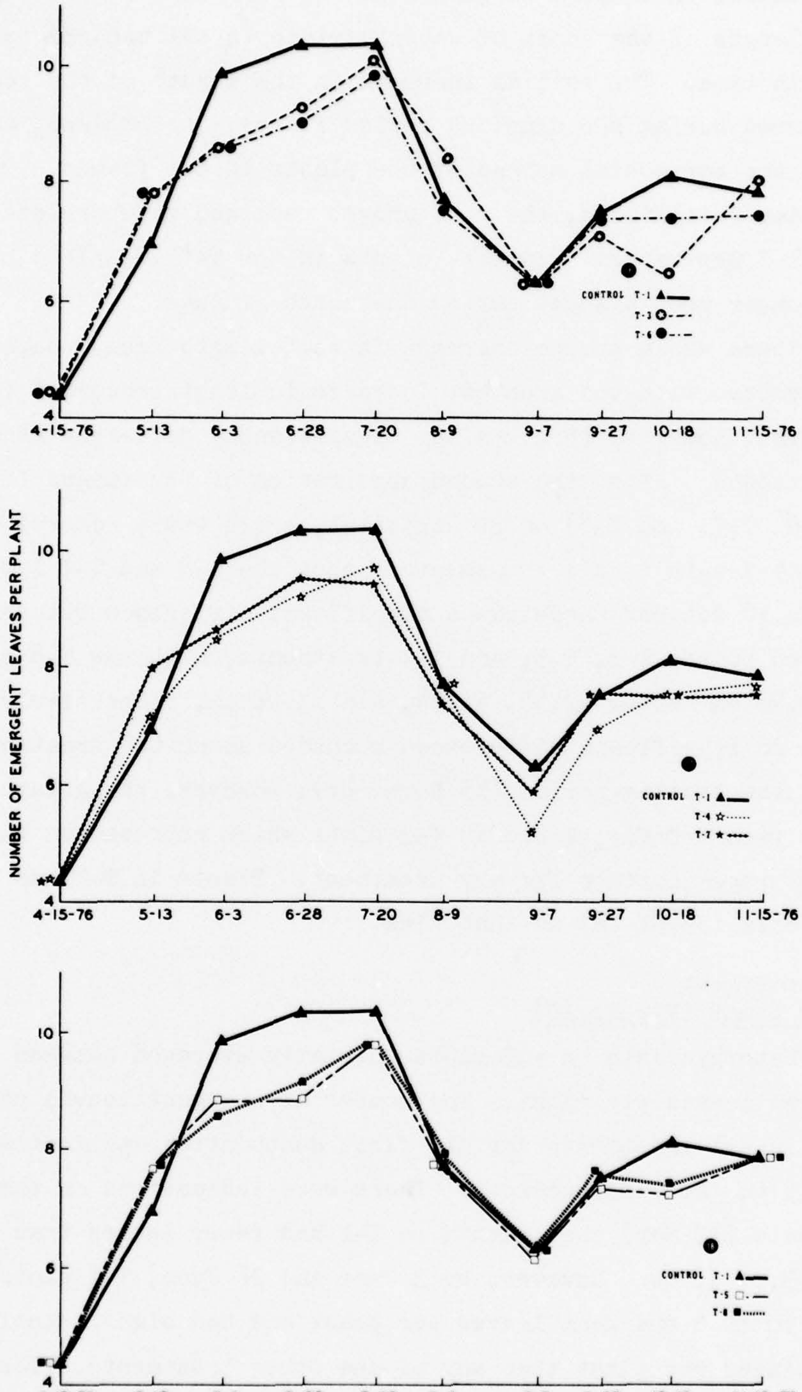


Figure 10. Number of emergent leaves per plant versus time

plant in the other treatments. These numbers declined to minimal values by 7 September. There were indications that plants in T-4 had the least number of emergent leaves per plant, whereas plants in T-1 had the highest number.

22. The number of leaves increased in all treatments over the next three sampling periods (27 September to 15 November) with no significant differences noted except that there were indications (0.09) that plants in T-1 had more emergent leaves than those in T-3 and T-5 on 18 October. On the last sampling date (15 November), although there were no significant differences in treatments, there were indications that all treatments, except T-3, had fewer emergent leaves per plant than T-1. Plants in T-3 plots had significantly more emergent leaves per plant than those in T-6 and T-7 plots. Inoculum levels T-6 and T-7 had fewer emergent leaves than their basic inoculum levels T-3 and T-4 (7.48:810 and 7.60:7.74, respectively). The number of emergent leaves in T-5 and T-8 was similar (7.83:7.84, respectively).

Total damage per plant
(emergent leaves) (Figure 11)

23. *Cercospora rodmanii* was applied to the plants in the designated plots when the plants averaged approximately 4.5 emergent leaves; therefore, only a small number of leaves actually received the initial inoculum. At the beginning of the test, there was a resident damage on the original plants due to natural infection. Additional damage to emergent leaves of waterhyacinth was apparent approximately 2 weeks after the application of *C. rodmanii* to the plants. There was a highly significant difference (0.01) in the total damage to the emergent leaves per plant for all treatments relative to T-1 on 13 May and 3 June, with the highest damage present on plants in T-(5,8) (30.21) and the least damage on plants in T-1 (18.11). There was a sharp increase in total damage to plants in T-1 plots during the next sampling period (28 June) and, although there were no significant differences among inoculated treatments, there was a significant difference in damage per plant for T-1 when compared to plants in T-(5,8) and T-(3,6) plots.

24. Maxima for total damage per plant were reached on 20 July in

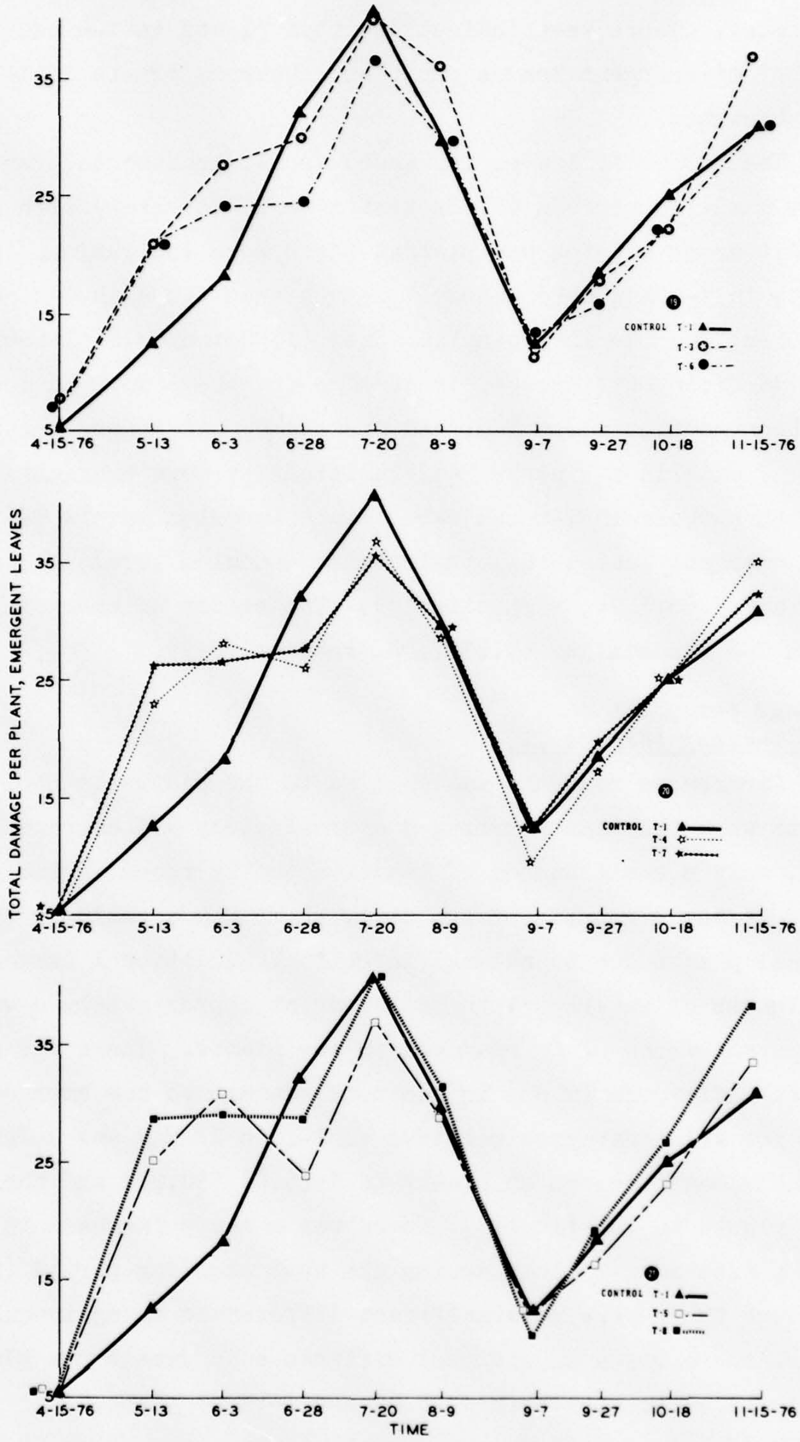


Figure 11. Total damage per plant (emergent leaves) versus time

all treatments and controls; there were indications that damage was greatest in T-1 plots compared to plants in T-7. All plots showed a decrease in total damage until 7 September when damage to plants rapidly increased in all plots for the duration of the test. Although there were no significant differences in total damage among treatments on 15 November, there were indications (0.1) that the greatest damage per plant occurred on the emergent leaves in the T-8 plots. The least damage per plant was recorded for plants in T-1 and T-6. In comparison to the basic inoculum levels, only plants in the T-8 plots showed an increase in damage of those plots receiving a second application of the fungus.

Total damage per
emergent leaf (Figure 12)

25. The resident damage on the emergent leaves at the beginning of the test was approximately 1.2 and, according to the rating scale used, this represented only a few lesions per leaf. However, there were indications that plants in T-(5,8) plots had higher damage ratings than plants in T-(4,7). Data collected on the second and third sampling dates (13 May and 3 June) showed that a highly significant difference existed between all treatments compared to T-1, which showed the least damage. In addition, a highly significant difference (0.01) existed on 13 May between T-(5,8) and the T-(3,6) treatments, with the T-(5,8) having more damage per leaf. There was no significant difference between the T-(5,8) and T-(4,7) inoculum levels at that time. There were no further differences among treatments until after the second application of the fungus to the plants on 30 September.

26. Damage per emergent leaf increased until 20 July when maximum values were recorded for T-1, T-4, T-5, and T-8. Maximum values were recorded for T-3, T-6, and T-7 plots on 9 August. There was a rapid decrease in damage for all treatments and T-1 on 7 September. On the first sampling date after the application (18 October), there was a highly significant difference (0.01) in damage between T-8 compared to the T-3 and T-6 treatments and a significant difference (0.05) between T-8 and T-1, with the greatest damage per leaf occurring in the T-8

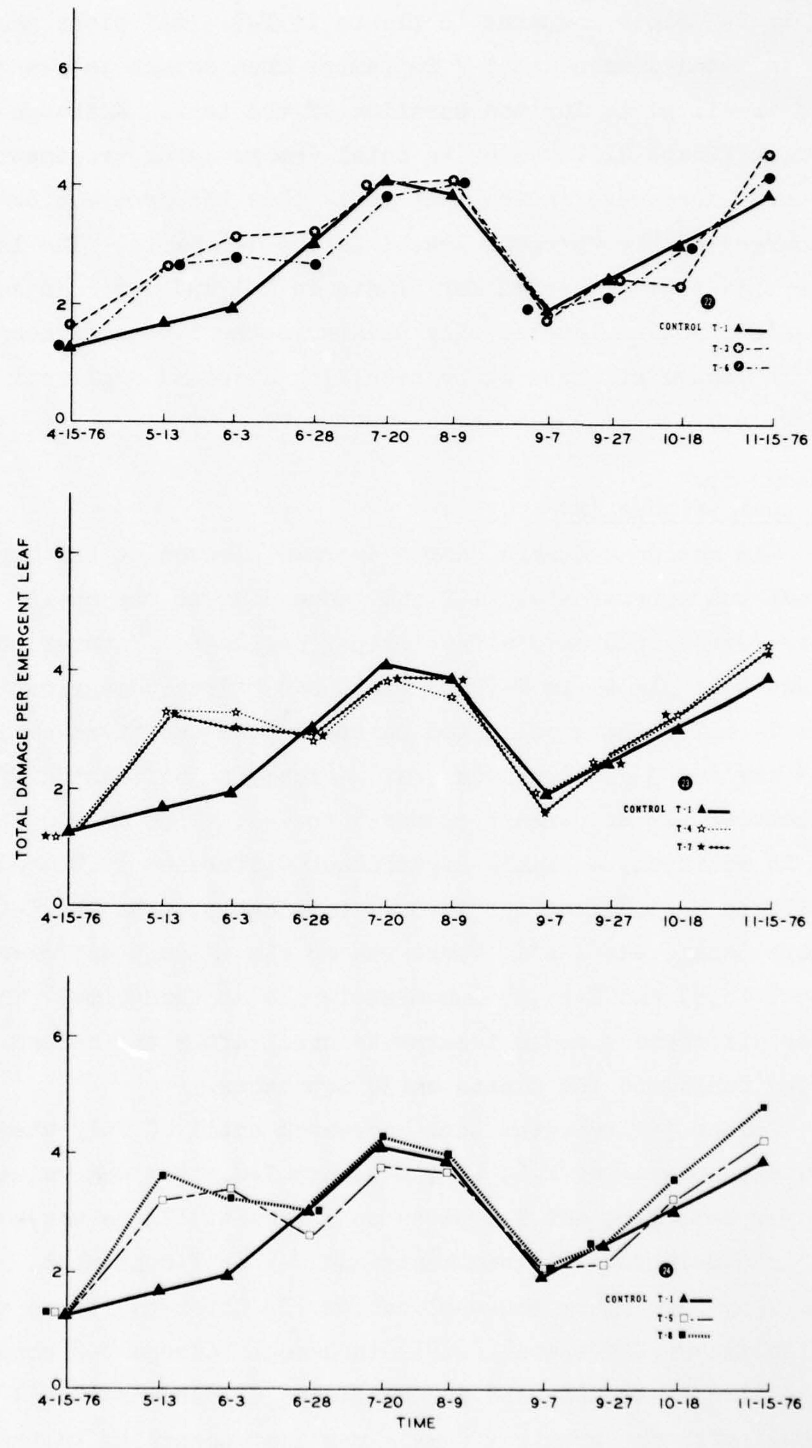


Figure 12. Total damage per emergent leaf versus time

plots. Although there were no significant differences on the last sampling date (15 November), there was an indication (0.1) that greater damage per emergent leaf of waterhyacinth occurred in the T-8 plots compared to T-1. Of those plots receiving a second application of the fungus, only T-8 showed an increase in damage per emergent leaf compared to the basic inoculum levels. However, emergent leaves in all treated plots were assessed higher damage ratings compared to those in T-1 plots.

Rate of increase in the number of
emergent leaves per plant (Figure 13)

27. Sampling dates 15 April-3 June. Using a simple regression equation, the rate of increase in the number of emergent leaves per plant, which is a measurement of the slope B_i , was determined. The equation used was:

$$Y = B_o + B_i X \tag{1}$$

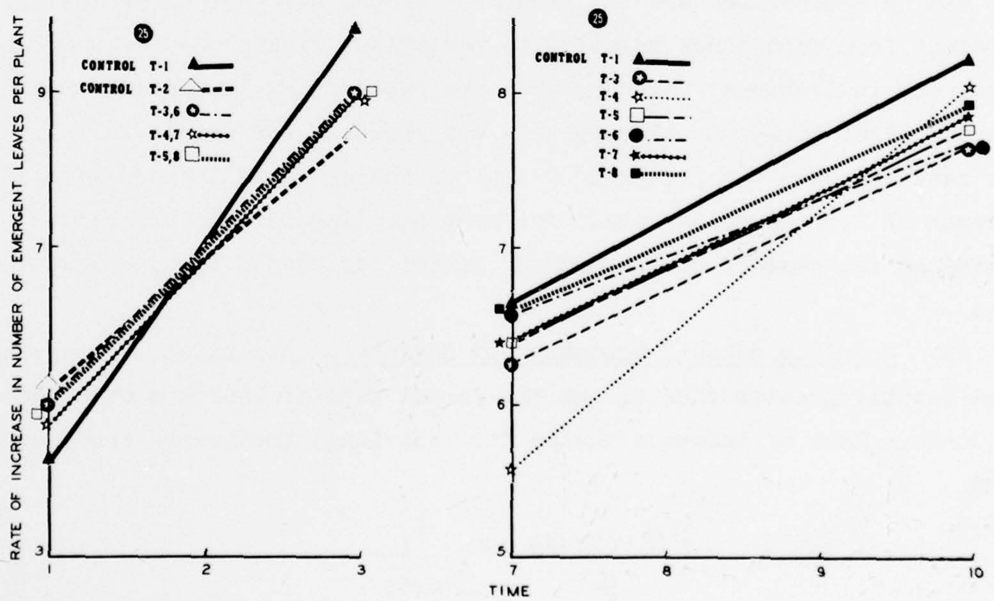


Figure 13. Rate of increase in number of emergent leaves per plant versus time

where

Y = number of emergent leaves per plant

B_o = initial number of emergent leaves per plant

B_i = rate of increase in the number of emergent leaves per plant

X = time, days

Similar inoculum levels were combined for the computation (i.e., T-(3,6), T-(4,7), and T-(5,8)) because they represented the same inoculum level at that time. The rate of increase values for each basic inoculum level are given in the following tabulation:

Treatment	B_i
T-1	2.822
T-(3,6)	2.048
T-(4,7)	2.113
T-(5,8)	2.108

28. All regression line slopes are significantly greater than 0, and the common rate of increase for the four lines is 2.194. The common rate was determined because the treatment slopes were not significantly different from each other relative to variations within the treatments. There were indications, however, that the rate of new leaf production over the first three sampling periods was greatest for plants in T-1. This rate increase for plants in T-1 plots indicated that there was an increase of 2.8 leaves per plant for each sampling period compared to an average increase of 2.1 leaves per period for plants in the treated plots.

29. Sampling dates 7 September-15 November. All rates were again significantly greater than 0, and the common rate of increase or slope was 0.496. Rate of increase values for individual treatments are shown below.

Treatment	B_i
T-1	0.537
T-3	0.480

(Continued)

Treatment	B_i
T-4	0.842
T-5	0.491
T-6	0.380
T-7	0.512
T-8	0.458

Each rate was recorded separately to reflect the effect of the second application of *C. rodmanii* to treatments T-6, T-7, and T-8. During the last four sampling periods, the rate of new leaf production for T-1 was 0.54 leaves per plant for each period and, for the treated plots, varied from 0.38 to 0.84 leaves per plant for each period. It was evident from these slopes that the rate of leaf production had decreased considerably when compared to the rates for the first three sampling periods.

30. Even though there were no significant differences in rates among the treatments, the highest rate of increase in emergent leaves per plant occurred in the T-4 treatment and the lowest rate in the T-6 treatment. There was a tendency for the rates of emergent leaf production to be lower for plants in plots that received a second application of *C. rodmanii* when compared to their basic rates (i.e. T-6 versus T-3).

Rate of increase of damage per plant (emergent leaves) (Figure 14)

31. Sampling dates 15 April-3 June. Similar treatments were combined for analysis because they represented the same basic inoculum levels. All treatments, except T-2, were significantly greater than 0 and had a combined rate increase for damage per plant of 10.452. The rate increase values for combined treatments are given below:

Treatment	B_i
T-1	6.348
T-2	0.301
T-(3,6)	9.952
T-(4,7)	11.333
T-(5,8)	12.124

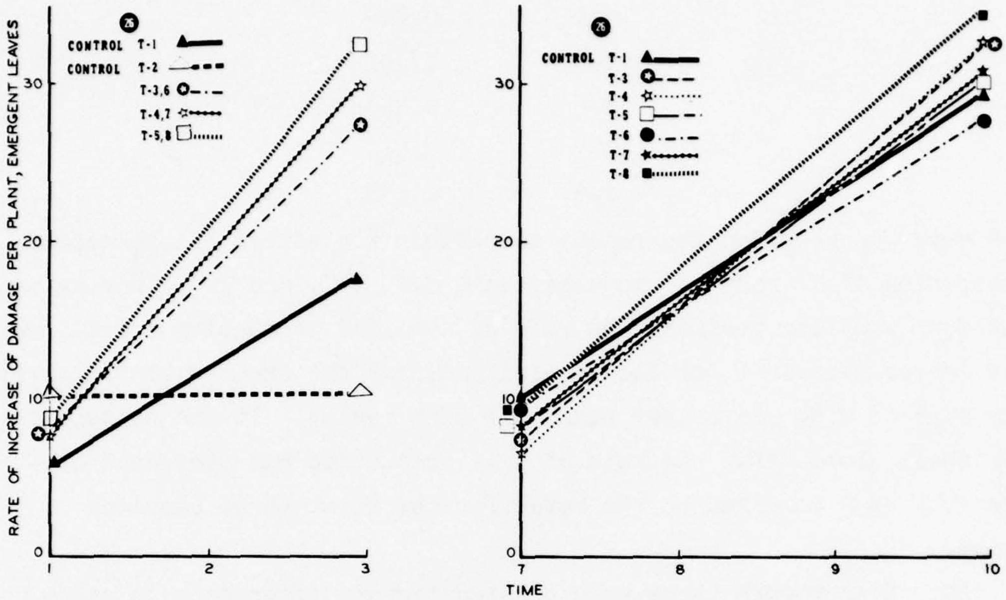


Figure 14. Rate of increase of damage per plant (emergent leaves) versus time

32. Although there were no significant differences among the basic inoculum levels, there were indications that an increase in damage on the emergent plant parts occurred with increasing inoculum levels during this time period. According to the rating system used, a slope of 6.348 for T-1 plots indicated that for each sampling period one new leaf was infected and that the damage on this leaf was equivalent to a rating of at least 6 (see Table 2). This leaf would be characterized by many lesions on the leaf blade and petiole and one third of the leaf blade would be necrotic. Damage per plant at the highest inoculum levels would be characterized by the death of one leaf with a second leaf damaged so that one half of the leaf blade was covered with fungal lesions. During the next three sampling periods, 3 June-20 July, the rate increase for T-1 was greatest and the slope of damage per plant was 12.425. Slopes for the increase of damage for treated plots decreased to 6.37, 4.58, and 4.52 for T-(3,6), T-(4,7), and T-(5,8), respectively. The slope for the fungicide treated plots, T-2, indicated that the disease was increasing very slowly on these plants and was being

controlled by the application of the fungicides during this period.

33. Sampling dates 7 September-15 November. All rates were significantly greater than 0 and had a common slope of 7.475. There were no significant differences among individual treatments and the rate values are listed below:

Treatment	B_i
T-1	6.475
T-3	8.419
T-4	8.774
T-5	7.298
T-6	6.158
T-7	7.619
T-8	8.546

However, the data indicated that the greatest increase in damage during this period occurred on plants in the T-4 treatment and that the least increase was on plants in the T-6 treatment. The T-8 treatment was the only treatment of those that received a second inoculation that showed a greater increase in damage when compared to its corresponding basic rate (i.e. T-8 versus T-5). The rate of damage to plants in T-1 represented the second lowest rate increase of the treatments. Therefore, according to the rating system used, damage per plant in T-1 for the last four sampling periods was equivalent to the amount of damage received during the first three sampling periods. Damage to plants in the treated plots would be slightly less than the first periods, but would still result in the loss of the photosynthetic leaf surface of one leaf per sampling period.

Rate of increase of damage
per emergent leaf (Figure 15)

34. Sampling dates 15 April-3 June. The average damage for each emergent leaf increased in all plots during this period. The rate of increase values for each of the treatments, except T-2, was significantly greater than 0, and the common rate of increase for all treatments, except T-2, was 0.868. There were no significant differences

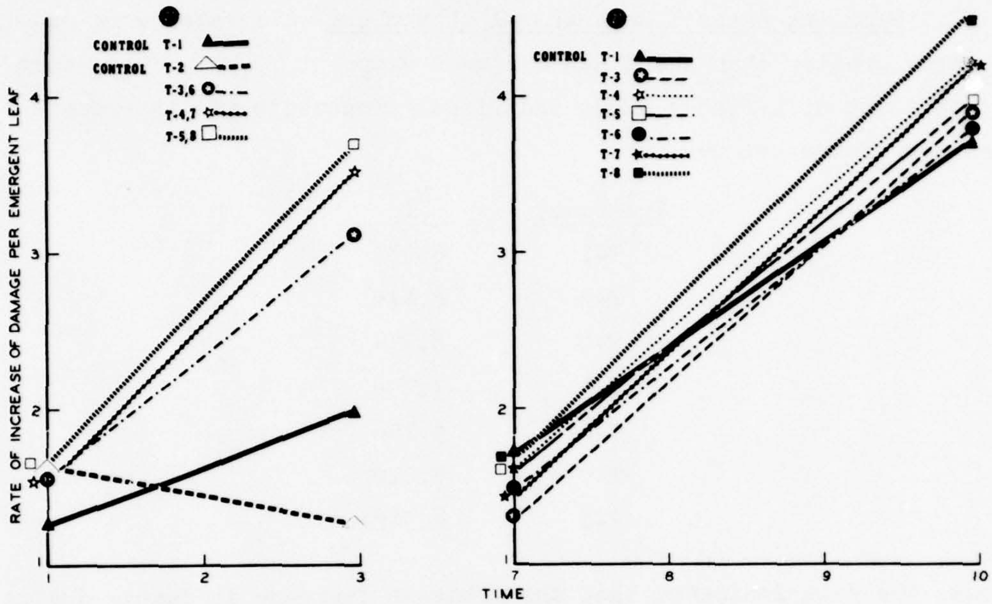


Figure 15. Rate of increase of damage per emergent leaf versus time

among treatments due to the variation within individual treatments. The rates of increase for the combined treatments and fungicide-treated controls are listed below:

Treatments	B_i
T-1	0.365
T-2	-0.180
T-(3,6)	0.794
T-(4,7)	1.022
T-(5,8)	1.038

35. According to the rating scale used, each leaf per plant in T-1 plots increased by 0.365 units of the scale for each period. For instance, if each leaf were rated as 1.0 in the scale at the beginning of the test, then by the next sampling date each leaf would have an average damage of 1.365 which corresponds to an increase in lesion coverage of the leaf surface. Likewise, leaves on plants in T-(5,8) would average an increase of one unit of the rating scale per sampling period.

Therefore, there were indications that an increase in damage per emergent leaf was present with increasing inoculum levels.

36. Sampling dates 7 September-15 November. All rates of increase of damage per emergent leaf were significantly greater than 0, and the common rate of increase for all treatments was 0.833. There were no significant differences between treatments, but there were indications that damage to emergent leaves was greater at the higher levels of inoculum compared to T-1. The highest rates of increase were recorded for the T-8 and T-7 inoculum levels. The lowest rate of increase in damage per emergent leaf was recorded for plants in the T-1 plots. The individual rates are listed below:

Treatment	B_i
T-1	0.674
T-3	0.874
T-4	0.885
T-5	0.799
T-6	0.779
T-7	0.934
T-8	0.971

These data indicated that change in disease symptoms was greatest in the inoculated plots when compared to T-1. Treatments T-7 and T-8 were the only treatments that received a second inoculation that showed an increase in slope compared to the basic inoculum levels.

Fungicide control of *C. rodmanii* on waterhyacinth

37. Waterhyacinth in plots that were treated with fungicides were rated on the same schedule as the other treatments. Based on initial treatments with the fungicides, Daconil 2787 did not appear to be as effective in controlling *C. rodmanii* as Benlate. Therefore, the alternation of fungicides to avoid the development of a fungicide-resistant strain of the fungus was discontinued after 13 May in order to achieve better control of the disease using Benlate alone. The efficacy of the fungicide was apparent from the data (Figures 14 and 15). The progress

of the disease on emergent leaves of waterhyacinth over the first three sampling periods in plots treated with the fungicide was characterized by a negative slope (-0.180). Ratings of damage in these plots were discontinued after the fifth (20 July) sampling date, however, because of extremely high mite populations which interfered with disease assessment.

Visual assessment
of disease progress

38. Visual assessment of disease progress and other factors that may have contributed to disease progress were recorded to supplement the data that were collected. A brief chronology of observations that will be discussed later in relation to the numerical data presented in the Results section is presented in the following paragraphs.

39. 15 April. Plants in all the frames appeared similar with waterhyacinth covering approximately 1 m^2 of the enclosed area of each frame (Figure 4).

40. 13 May. The coverage of the waterhyacinth in each frame was estimated to be 1.5 m^2. No treatment showed a consistent reduction in waterhyacinth coverage. There was a marked difference in initial infection noted and the higher inoculum levels could easily be separated from the other treatments. The waterhyacinth in the fungicide-treated plots showed a slight burn on the leaf tips. The maximum-minimum temperature records for the air at canopy level and the water at the root level showed a close correlation with only a few degrees difference.

41. 26 May. Mite infestations were noted on waterhyacinth in many of the plots. There appeared to be a greater number of offshoots in the treated plots compared to the untreated controls (T-1). Waterhyacinth in each plot had been producing new leaves at a rate of one leaf every 5 to 6 days; therefore, the upper canopy of leaves appeared green (Figure 16). However, damage to the older leaves was still evident and the greater symptom expression occurred at higher inoculum levels. *Cercospora rodmanii* symptoms were present on the new leaves and also on the offshoots (Figure 17) which indicated that the fungus had spread via conidia to the uninfected plant tissue.

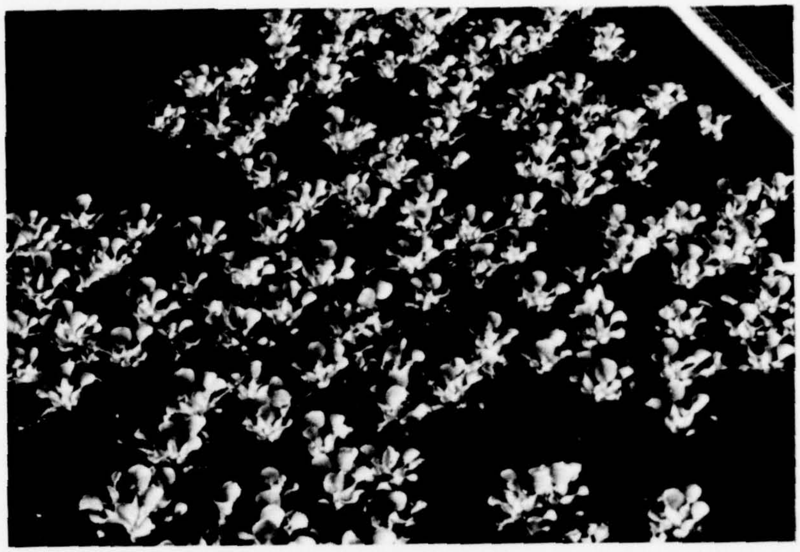


Figure 16. Waterhyacinth inoculated at the T-(4,7) level showing an upper canopy of healthy leaves with the older leaves infected with *C. rodmanii*, 3 June 1976

Figure 17. A close-up of a T-(4,7) plot showing the presence of *C. rodmanii* on the older leaves of a mother plant (M_1) and the spread of the disease to its offshoot (M_2), 3 June 1976

42. <u>28 June.</u> Waterhyacinth covered 5 to 6 m^2 of the enclosed area of the frames in all treatments. Offshoot production appeared to be greater in treated plots compared to T-1, but not at a high enough rate to significantly influence the total coverage. Mite infestations were evident in most plots.

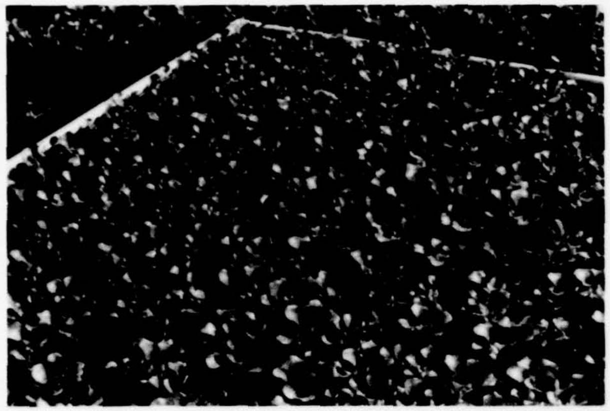
43. <u>20 July.</u> Waterhyacinth coverage of the enclosed areas of most frames was approximately 8 m^2. Mites were still present in most of the plots. Some of the original plants had died due to the disease. The disease appeared to be well established on plants in all of the plots except the fungicide-treated controls (Figure 18).

44. <u>9 August.</u> All frames were completely full of waterhyacinth. Disease severity appeared to be similar on waterhyacinth in all of the frames. The fungicide-treated plants were heavily infected with mites and appeared burned. The red coloration of the plants due to the mites readily distinguished the fungicide-treated plots from the other treatments in the test.

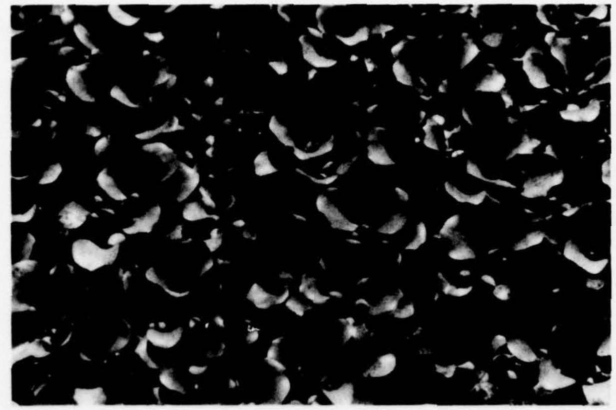
45. <u>7 September.</u> When chains of offshoots were removed from the plots, the last plant (oldest) in the chain was usually dead or severely damaged. This last plant was probably one of the original plants that had been stocked in the frames at the beginning of the test. Most of the growth of the waterhyacinth, which had been horizontal via offshoots, was now directed vertically and had resulted in an increase in the height of the plants in all of the plots. The severity of the disease on the plants appeared to be similar in all plots except the fungicide-treated plots.

46. <u>27 September.</u> The leaves of waterhyacinth in the upper canopy showed no symptoms of *C. rodmanii* (Figure 19). There was a noticeable increase in the height of the plants in all of the plots. Disease symptons on the plants appeared to be similar in all treated plots, except the fungicide treatment. The length of the roots of the plants in all treatments had greatly increased.

47. <u>18 October.</u> Waterhyacinth in plots that received the second application of *C. rodmanii* showed an increase in sympton expression compared to the plants that had received only the initial application



a. T-2



b. T-(3,6), note the dead mother plant (M_1)

c. T-(4,7)

d. T-(5,8), note the spread of the disease from the mother plant (M_1) to the offshoots

Figure 18. A comparison of disease severity of *C. rodmanii* at the different inoculum levels, 20 July 1976

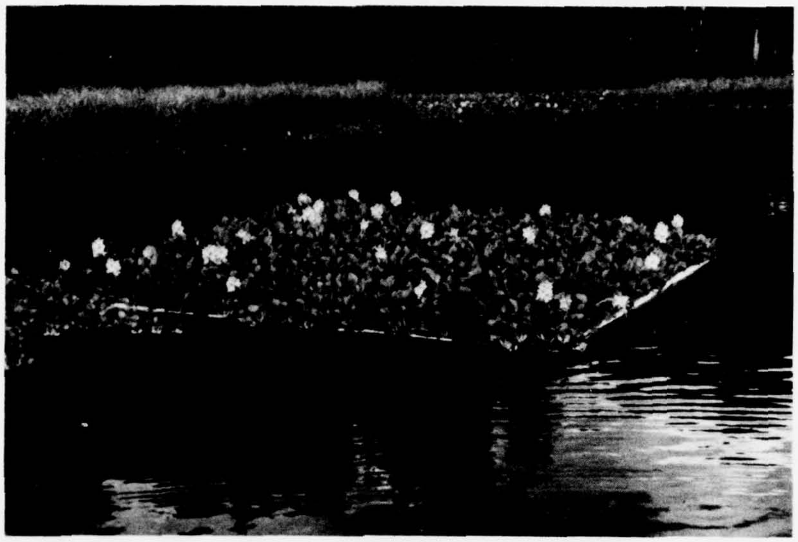


Figure 19. The green canopy effect was noted in most waterhyacinth plots at this time and was characterized by this T-1 plot, 27 September 1976

(Figure 20). The greatest damage was noticed on waterhyacinth in T-8 and the least damage was noticed in T-1 and T-3. Most of the damage that occurred on plants in T-1 and T-3 was confined to the edges of the

Figure 20. Increase in disease severity was represented by this T-8 waterhyacinth plot. This increase in damage was due to the second application of *C. rodmanii* to plants in plots T-6, T-7, and T-8, 18 October 1976

plots. A heavy mite infestation was present on waterhyacinth in all fungicide-treated plots. Populations of waterhyacinth in the lake surrounding the frames were severely infected with *C. rodmanii* and were exhibiting decline symptoms.

48. 15 November. The first frosts occurred on 6 and 9 November and the tips of the larger leaves in most of the plots exhibited a frost burn; however, most of the tip dieback of blade and petiole was caused by *C. rodmanii* (Figure 21). The frost damage was confined to the edges of the plots.

Discussion

Reliability

49. The ability to assess the effect of *C. rodmanii* on waterhyacinth was severely hampered by the loss of reliability of the fungicide-treated controls. Without a baseline with which to compare damage in the treatments, all comparisons had to be made with the untreated

Figure 21. Increased damage of waterhyacinth by *C. rodmanii* in T-8 plots was characterized by leaf and petiole necrosis. Damage to some leaf tips had been compounded by frost, 15 November 1976

controls, T-1. However, the cross-infection of plots by *C. rodmanii* limits valid comparison with T-1 plots to a period of 4 to 6 weeks after application of the fungus. Values assessed to T-1 after this period of time would be increasingly influenced by the disease. This is supported by the data which show a lag period for T-1 followed by a rate of increase of damage on the waterhyacinth similar to the inoculated plots.

50. When the data from the results of the first three variables are examined, there are concomitant increases in the number of leaves per plant, total damage per plant, and the number of dead leaves per plant. All variables increased in value beginning with 15 April and reached maxima at 9 August or 7 September. The number of leaves per plant included a count of dead submerged leaves; this led to variations in count in the latter part of the experiment. The initial stock plants in the frames were more diseased than plants produced via offshoots. Many of the original plants had been killed and lost from the frames through submergence which resulted in the collection of data from offshoots that had not received the original inoculum. Furthermore, in the latter part of the test, sampling included a random selection of offshoots which may not have been as accurate a measurement of damage as sampling only the oldest plants. The older offshoots from the original stock also had a root rot and, when these plants were removed for measurement, the dead part of the root stock containing the dead submerged leaves broke off.

51. The death of the original mother plants, coupled with the random sampling of offshoots and the loss of the dead portion of the roots from some of the offshoots, resulted in a reduced count of total leaves, number of dead leaves, and assessment of total damage per plant during the latter part of the test.

Height of the plants

52. In this experiment the variable most affected by the presence of the disease was the height of the plants. This variable was measured as the longest leaf. The infection of the plants by the disease in the early part of the experiment had a significant effect on the height of the plants which was evident throughout the duration of the experiment

when compared to T-1. Although damage per plant (emergent leaves) in T-1 exceeded damage on inoculated plants on 20 July, the reduction in the height of the plants in T-1 was not as great as that which occurred on plants in the inoculated plots. It would appear from these data that the longer the disease stresses the plant the greater its effect.

Emergent leaves per plant

53. Subtracting the number of dead leaves per plant from the total number of leaves resulted in the new variable known as the number of emergent leaves per plant. The graph of this variable (Figure 10) showed that the number of emergent leaves reached maxima on 20 July. This corresponded to the visual observation on 9 August that the disease symptoms appeared similar in all plots. This increase, coupled with the rapid increase in the height of the plants that was beginning at that time, was responsible for the green canopy effect. Visually, the plots appeared green; as the leaves grew away from the inoculum source on the older leaves, the rate of the epidemic decreased. This decrease in damage to emergent leaves was evident in Figures 11 and 12 where damage peaked at 20 July and decreased for the next two sampling periods until 7 September. An increase in damage was evident on 27 September and was augmented on 30 September with the application of *C. rodmanii* to waterhyacinth in treatments T-6, T-7, and T-8.

Root length

54. Another variable that actively increased during the rapid formation of new leaves and their spatial separation from diseased leaves was the root length. The greater absorption area of these larger root systems probably compensated for the additional nutrient required for the more rapid growth of the plants during that part of the year.

Comparison

55. A comparison of the graphs of the four variables, number of dead leaves per plant, number of emergent leaves per plant, height of the plants, and total damage per plant (emergent leaves), reveals some interesting correlations concerning the progress of the epidemic. The highest recorded damage to emergent plant parts occurred on 20 July and 9 August and corresponded to the decrease in emergent leaves that was

recorded for 20 July through 7 September. This decrease represented a reduction of emergent leaves from an average of 10 leaves per plant to an average of 6, which resulted in a loss of sites for infection and sporulation when the leaves became submerged. The number of dead leaves per plant reached maximum values on 7 September, which is the same time that the number of emergent leaves reached minimum values (Figure 22). Therefore, it was during this time period that the greatest submergence of leaves occurred. This submergence probably resulted in a reduction in the rate of the epidemic, which is reflected in the decrease in the total damage to emergent parts of the plant. Finally, the rapid increase in the height of the leaves on the plants, which was occurring at that time, increased the distance between the inoculum source on the older

Figure 22. A waterhyacinth plant from plot T-(3,6) showing the relationship between the number of emergent leaves and the number of dead submerged leaves, 9 August 1976

leaves and the newly formed uninfected leaves, thereby creating a canopy effect. This might have also helped to reduce the rate of the epidemic.

56. The disease cycle was initiated again after 7 September and, as the number of emergent leaves increased, there was a concomitant increase in total damage per emergent part of the plant. Damage to the emergent parts increased rapidly; the assessed values per emergent leaf on 15 November were among the highest recorded during this test. Leaf damage due to frost appeared to be minimal at that time as only a few leaves per plot showed frost damage. The number of emergent leaves increased from six to approximately eight per plant, but appeared to be limited by the disease and probably to some extent by the cooler nighttime temperatures occurring at that time that slowed the growth rate of the waterhyacinth.

Fungicide control

57. The control of *C. rodmanii* with fungicides during the first three sampling dates (15 May-3 June) was generally good. Benlate appeared to perform better that Daconil 2787. The use of both fungicides produced a tip burn on the leaves of waterhyacinth. Baseline data concerning the rate of fungicide to be used to control disease on waterhyacinth were lacking and the tip burn was probably the result of using a higher concentration of the fungicide than needed. Another result of fungicide usage was the attraction to these treated plots of large populations of mites which remained throughout the duration of the test. These high mite populations imparted a red coloration to these plots and interfered with disease assessment on the plants.

Test termination

58. Although the frames were full of waterhyacinth at the end of the experiment, the total damage per emergent leaf had increased and was at the highest assessed value at termination of the test. These values indicated that the disease was severely infecting the plants and would probably provide a source of inoculum to initiate disease in the spring. In this regard, disease assessment during the next year, in March 1977, indicated that the disease had overwintered on the plants and that *C. rodmanii* was prevalent in all waterhyacinth plots. Unfortunately,

plans to continue this experiment for an additional year were interrupted due to a prolonged drought in the central Florida region that resulted in the loss of the entire volume of water from the lake.

Data analysis

59. The direct effect of *C. rodmanii* on waterhyacinth was analyzed over two periods of time following application of the fungus to the plants. These analyses were limited to the first three sampling dates and the last four sampling dates of the experiment; the results are presented in Figures 13-15. The first time period represents the initial effects of the fungus on the plants. Data after this period indicate that the disease spread and infected the untreated controls (T-1) as well as newly formed leaf material during the months of July and August. This spread of the disease also tended to equalize inoculum levels and eventually equalized the total damage in all plots. The last four time periods (7 September-15 November) reflect the buildup of a second epidemic and include the effect of the second application of the fungus to waterhyacinth in treatments T-6, T-7, and T-8.

60. According to the slope values derived from the data, the rate of increase of emergent leaves per plant was greater in all treatments during the first three sampling periods compared to the last four periods. During the first three periods plants in the untreated controls (T-1) produced approximately three leaves every sampling period compared to only 0.5 leaves per period during the latter part of the experiment.

61. The rate of increase of damage per emergent leaf in the untreated controls (T-1) was approximately twice as great during the latter part of the test than at the beginning (0.365 versus 0.674). This increase probably reflects the greater inoculum potential that existed on surrounding plants at the later period of time. Slopes did not vary greatly for the inoculated plots, indicating that the maximum rate of increase of damage established by the first inoculation could not be exceeded by either the direct application of a second inoculation or cross-infection from surrounding plots. This, however, should not be misconstrued to diminish the value of a second inoculation of the

fungus in the fall of the year because there are factors that also interfere with the assessment of the efficacy of this inoculation. One of these factors was the inability of the investigators to produce enough inoculum to treat the waterhyacinth in the designated plots (T-6, T-7, and T-8) with an amount of inoculum equal to the basic levels used at the beginning of the test. This resulted in the inoculation of only 5.3 g/m^2 of the fungus onto waterhyacinths in the T-6 plots. This amount of inoculum was not only less than the original levels, but it also had to cover taller plants with more leaves per plant than the original inoculation. Another factor that interfered concerned the ability of the fungus to spread to plants in other plots not directly inoculated with the fungus. This eventually resulted in the equalization of damage ratings on the plants by the end of the test when no significant differences were noted, except for T-8, among the various inoculum levels and T-1.

62. The similarities in rates during the beginning and end of the experiment for total damage per plant (emergent leaves) and total damage per emergent leaf indicated that the maximum rate was reached at inoculum level T-5 (196 g/m^2). Using the rating scale for disease assessment, a slope of 9.0 for total damage per plant (emergent leaves) indicated that on the average, during each sampling period, each plant had one of its uninfected leaves killed and submerged. If the corresponding rate increase in emergent leaves per plant is greater than 1.0, then the plant will be able to outgrow disease development and the canopy of the plot will consist of green leaves. However, when the rate of new leaf production falls below 1.C, such as it did during the last four sampling periods, the plant will undergo a decline. The death of the plant will depend upon the length of time that the plant is under these decline conditions.

Strategy

63. The strategy for use of the organism would, therefore, dictate that for maximum efficacy of *C. rodmanii* as a biological control of waterhyacinth the fungus should be applied when the growth rate of the waterhyacinth is low. This low growth rate occurs naturally in Florida

during the early spring and fall of the year, indicating that cooler temperatures affect the growth rate of waterhyacinth to the benefit of *C. rodmanii*.

64. In addition, the data indicate that it may be possible to maximize damage on waterhyacinth by applying the second application of the fungus to waterhyacinth during the month of June or July when the number of emergent leaves is at a maximum. However, the timing of application may vary depending on the environmental conditions that exist in the water body being treated. This application would possibly initiate an earlier epidemic in the fall and result in greater damage to the populations of waterhyacinth.

65. Another strategy exists with which to maximize damage of *C. rodmanii* on populations of waterhyacinth. This would utilize a growth regulator in combination with the fungus to retard the production of leaves before the peak of the waterhyacinth growth cycle, thus allowing the disease to infect all available leaves and severely affect further growth of the plant.

Conclusions

66. It has been shown that *C. rodmanii* can severely affect the growth of waterhyacinth, especially under conditions that favor a reduced growth rate for the plant. Therefore, environmental factors, such as temperature and availability of nutrients in the water, will affect the disease cycle and will determine when maximum damage to the disease will occur. In addition to the disease study, it has been determined that *C. rodmanii* can be controlled by the use of available fungicides.

67. The greatest effect of *C. rodmanii* on the waterhyacinth was a reduction of the height of the plants in comparison with the untreated controls. The direct application of the fungus onto the plants early in the year had more effect on height of plants compared to the indirect spread of the disease onto the untreated controls.

68. Plants that were not inoculated with the fungus directly can

be infected by secondary spread of the disease from the inoculated plots and after a period of time may exhibit as many disease symptoms as the inoculated plants. Therefore, one of the problems with assessing damage by a biological control organism such as *C. rodmanii* lies in the inability of controlling the fungus from spreading to infect plants in the untreated controls and to plots containing plants inoculated at various levels with the fungus. When these control plants become infected, a baseline comparison to accurately assess the progress of damage on the plants by the biological control cannot be made. Furthermore, when baseline data are not generated for comparison, this could possibly result in a bias against the efficacy of the biological control organism. In this regard, reductions in weed populations may be so subtle during the first years of its use that they may not be noticed. However, as populations of the biological control organism increase naturally or are manipulated by further inoculations, enough stress may be placed on the weed populations by the disease to significantly reduce weed population levels over a longer period of time. The spread of the disease to plants in other plots also interferes with the interpretation of rate studies designed to determine optimal levels of inoculation. However, this problem of the disease spreading to other plant populations and damaging those populations is in reality one of the criteria that indicates the usefulness of *C. rodmanii* as a biological control for waterhyacinth.

69. In the initial design of the test, the stocking of only 100 plants per frame and the application of the fungus onto only these plants might have favored the waterhyacinth relative to the disease. This is because direct infection was limited to only those leaves inoculated (approximately 400 per frame); when the original plants began to produce offshoots, new plants that had not recieved any inoculation were included in the plots. Therefore, the original plants were allowed to outgrow the disease vertically through new leaf production as well as horizontally via offshoots. There was only 1 m^2 of waterhyacinth in the plots during the first inoculation. However, the disease had to become established on these plants and ultimately spread to infect an

area of waterhyacinth nine times greated than the original coverage. Future tests should take this into account and utilize smaller frames so that they can be fully stocked and all plants can be initially inoculated.

Recommendations

70. It is recommended that this research continue and that future field testing be modified in the following ways:

a. The inoculum level T-4 and T-7 (96 g/m^2) should be dropped and only the high and low levels should be tested further. This will relieve some of the burden of data collection. However, it is also recommended that the number of replications be increased from four to six.

b. The size of the frames should be reduced to eliminate some of the variability that was seen within treatments. This can be easily accomplished by cutting the existing sides of the frames in half and purchasing new corner fittings. The area of enclosure will be reduced from 9 m^2 to 2.25 m^2. This reduction will increase efficiency of sampling and increase uniformity of coverage during application of the fungus.

c. The frames should be stocked full of plants at the beginning of the experiment. The free-floating plants produced a great number of offshoots and only a small number of plants per frame were actually treated. It is felt that confinement and treatment of all plants will reduce greatly the variability within treatments.

d. In order to more rapidly evaluate the efficacy of the fungus to initiate infection, a new technique, which has been developed at the University of Florida, Department of Plant Pathology laboratory, should be employed. This consists of tagging the oldest and newest leaves of the plants in a subpopulation of plants in the frames prior to treatment with the fungus. The damage is then recorded only on those leaves which directly received inoculum during the application. Sampling should be done biweekly until the newest leaf becomes submerged.

e. It should no longer be necessary to randomly arrange the frames on one line. To reduce the possibility of cross-infection of the disease, it is recommended that similar treatments be positioned together and that different treatments be spatially separated as much as possible in the lake.

71. It is also recommended that, once a constant source of inoculum is established, the field testing program be expanded (keeping in mind possible Environmental Protection Agency regulations). This expanded program should utilize the authors' system of evaluation to determine the efficacy of the *C. rodmanii* product. Data from these tests should be incorporated into a computer to develop a management system for the disease which will allow for maximum damage to waterhyacinth populations under varying environmental conditions.

References

Conway, K. E. 1976. "Evaluation of *Cercospora rodmanii* as a Biological Control of Waterhyacinths." Phytopathology, Vol 66, pp 914-917.

Conway, K. E., and Freeman, T. E. 1976. "Field Evaluations of *Cercospora rodmanii* as a Biological Control for Waterhyacinths." Proc. Amer. Phytopathol. Soc., Vol 3, p 272.

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Table 1

Data Values for Each Variable Used for the Graphs

Treatment Time	T-1	T-3	T-4	T-5	T-6	T-7	T-8
				Number of Leaves Per Plant			
1	4.20	4.70	4.43	4.53	4.48	4.38	4.58
2	7.42	8.43	8.00	8.35	8.30	8.43	8.46
3	11.20	10.75	10.33	10.80	10.87	10.90	10.90
4	12.90	13.40	13.03	13.60	14.17	13.80	13.90
5	15.70	16.38	16.28	16.60	16.23	16.27	16.85
6	19.35	19.88	20.13	19.43	18.53	19.20	18.95
7	18.57	20.08	18.95	20.93	20.80	19.57	18.40
8	15.55	13.55	14.58	15.38	14.90	14.10	15.30
9	14.60	13.70	13.80	14.30	13.70	14.27	14.10
10	14.75	15.90	14.83	13.73	13.27	14.07	14.78
				Number of Dead Leaves Per Plant			
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.38	0.53	0.80	0.63	0.33	0.67	0.68
3	1.39	2.05	2.23	2.13	2.20	2.20	2.28
4	3.42	4.20	4.35	4.85	5.03	4.74	4.63
5	5.92	6.26	6.64	6.53	6.96	7.07	7.29
6	12.12	11.70	12.85	12.05	11.77	12.20	11.55
7	12.68	14.10	14.55	15.25	15.17	14.70	13.10
8	8.25	6.75	8.13	8.58	8.19	7.07	8.37
9	6.83	7.98	6.96	7.40	7.13	7.50	7.09
10	7.18	7.80	7.45	6.68	6.20	6.40	7.40
				Total Damage Per Plant			
1	5.16	7.80	4.83	6.03	5.20	4.95	5.90
2	15.87	25.83	30.33	30.73	24.17	31.77	34.92
3	30.30	46.73	47.05	49.40	44.33	46.58	49.83
4	57.00	67.43	63.46	66.98	70.13	66.53	70.38
5	92.54	96.05	95.48	100.23	95.77	98.60	104.45
6	137.33	139.20	142.45	135.65	132.50	137.97	133.60
7	125.54	137.55	139.15	148.58	148.57	140.37	129.38
8	92.32	77.85	88.88	89.53	85.93	81.93	99.65
9	85.08	90.93	84.23	88.70	83.73	91.30	88.03
10	93.48	108.00	101.33	90.70	85.30	91.36	103.15

(Continued)

Table 1 (Continued)

Treatment Time	Treatment						
	T-1	T-3	T-4	T-5	T-6	T-7	T-8
				Height of Plants, cm			
1	13.86	14.28	13.60	13.08	13.88	13.93	12.83
2	11.87	14.25	9.98	10.68	11.83	9.93	11.25
3	10.40	10.35	9.28	11.35	9.73	9.75	10.48
4	10.11	9.73	10.00	10.20	10.40	10.00	10.40
5	10.94	10.65	10.10	10.85	10.95	9.46	9.93
6	15.73	12.93	12.24	12.85	13.38	11.31	12.88
7	24.09	20.09	17.63	20.59	20.48	20.13	20.34
8	30.91	27.83	25.78	27.99	27.83	27.95	27.80
9	34.67	29.74	27.81	28.62	32.39	31.19	32.31
10	31.92	28.68	25.59	27.81	27.95	27.30	30.57
				Length of Roots, cm			
1	16.01	15.33	12.63	15.00	13.08	11.40	14.53
2	25.53	27.65	25.40	24.58	26.97	24.93	27.04
3	38.07	38.78	35.53	37.21	33.97	38.25	40.20
4	48.00	50.86	47.80	47.55	49.17	45.47	52.40
5	50.70	53.03	54.30	51.45	49.31	45.77	53.20
6	49.70	48.09	47.55	48.00	40.09	48.86	52.19
7	49.75	46.87	48.75	48.75	45.78	44.57	48.97
8	60.15	60.03	56.90	56.96	66.79	53.59	63.69
9	56.59	49.35	56.95	51.84	55.48	53.90	62.48
10	59.56	64.08	57.81	58.13	57.77	55.91	61.47
				Number of Emergent Leaves Per Plant			
1	4.33	4.70	4.39	4.53	4.47	4.40	4.58
2	7.00	7.90	7.20	7.73	7.97	8.04	7.78
3	9.98	8.70	8.55	8.91	8.67	8.70	8.63
4	10.46	9.41	9.33	8.97	9.13	9.61	9.28
5	10.48	10.23	9.83	9.90	9.93	9.54	9.92
6	7.76	8.72	7.83	7.77	7.58	7.49	8.04
7	6.32	6.28	5.14	6.14	6.26	5.89	6.22
8	7.56	7.15	6.96	7.39	7.41	7.57	7.69
9	8.17	6.49	7.58	7.22	7.54	7.55	7.42
10	7.91	8.10	7.74	7.83	7.48	7.60	7.84

(Continued)

Table 1 (Concluded)

Treatment Time	Treatment T-1	T-3	T-4	T-5	T-6	T-7	T-8
			Total Damage Per Plant (Emergent Leaves)				
1	5.41	7.80	4.93	6.03	5.20	5.08	5.90
2	12.47	21.10	23.13	25.10	21.17	26.60	28.71
3	18.11	28.28	28.56	31.07	24.53	26.78	29.35
4	32.81	30.33	26.31	23.78	24.83	27.99	28.75
5	42.96	41.02	37.48	37.31	37.27	36.24	41.16
6	30.39	36.64	28.81	28.83	29.76	29.92	32.16
7	12.30	11.18	9.40	12.32	13.41	9.68	13.34
8	18.74	18.02	17.08	16.32	15.72	19.72	19.32
9	25.64	22.35	25.36	23.31	22.49	25.44	27.07
10	31.59	37.80	35.89	34.31	31.67	33.17	39.25
			Total Damage Per Emergent Leaf				
1	1.23	1.63	1.09	1.28	1.16	1.15	1.26
2	1.69	2.63	3.32	3.28	2.67	3.28	3.70
3	1.96	3.18	3.31	3.42	2.79	3.02	3.28
4	3.13	3.25	2.80	2.63	2.67	2.94	3.08
5	4.19	4.09	3.93	3.86	3.88	3.96	4.38
6	3.96	4.19	3.62	3.76	4.07	3.98	4.06
7	1.95	1.74	2.00	2.10	1.97	1.64	2.13
8	2.55	2.44	2.47	2.15	2.12	2.53	2.51
9	3.14	2.31	3.34	3.27	2.95	3.34	3.69
10	4.00	4.70	4.66	4.39	4.29	4.48	4.97

Table 2

Rating Scale System for Damage to Leaves of Waterhyacinth by *Cercospora rodmanii*

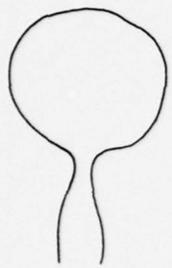
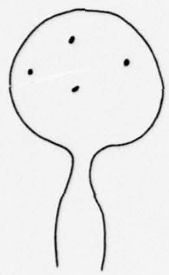
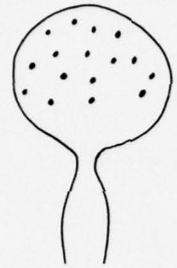
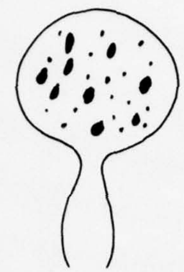
Numerical Ratings and Symptoms				
0	1	2	3	4
No spots on leaf or petiole.	1 to 4 spots on leaf, no petiolar spotting.	Less than 25 percent of leaf surface with spots, no coalescence or petiolar spotting.	Less than 50 percent of leaf surface with spots, some coalescence, no petiolar spotting.	Less than 25 percent of leaf surface with spots, coalescence, some tip dieback and petiolar spots.
				
5	6	7	8	9
Less than 50 percent of leaf surface with spots, coalescence, 10 percent tip dieback, petiole spotting.	Less than 75 percent spots, coalescence, 30 percent tip dieback, increasing petiole spotting.	Greater than 75 percent spots, coalescence, 60 percent tip dieback, coalescing spots on petiole.	Dead leaf blade, petiole green, but heavily spotted.	Dead leaf blade and petiole (submerged).
				

Table 3

Significant Responses of Treatments

Variable	Sampling Date, 1976*									
	4/15	5/13	6/3	6/28	7/20	8/9	9/7	9/27	10/18	11/15
Number of leaves per plant					(5,8)					
Number of dead leaves per plant			All	All	All					
Total damage per plant	(3,6) (5,8)	All	All	All	(5,8)					
Height of leaves					1	1	1	1	1	1 8
Length of roots	1	(3,6)	(5,8)	(5,8)					8	
Number of emergent leaves per plant		(3,6)	1	1			1		1	3
Total damage per plant (emergent leaves)		All	All	1						8
Total damage per emergent leaf	(5,8)	All (5,8)	All						8	8

* All = All inoculum levels significantly greater than T-1. For a description of these significant responses, see the Results section of the text.

In accordance with letter from DAEN-RDC, DAEN-ASI dated 22 July 1977, Subject: Facsimile Catalog Cards for Laboratory Technical Publications, a facsimile catalog card in Library of Congress MARC format is reproduced below.

Conway, K E
Field evaluation of Cercospora rodmanii as a biological control of waterhyacinth; inoculum rate studies / by K. E. Conway ... [et al.], Department of Plant Pathology, University of Florida, Gainesville, Florida. Vicksburg, Miss. : U. S. Waterways Experiment Station ; Springfield, Va. : available from National Technical Information Service, 1979.
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References: p. 46.

1. Aquatic plant control. 2. Biological control. 3. Cercospora rodmanii. 4. Florida. 5. Fungi. 6. Fungicides. 7. Inoculation. 8. Water hyacinths. I. Florida. University, Gainesville. Dept. of Plant Pathology. II. United States. Army. Corps of Engineers. III. Series: United States. Waterways Experiment Station, Vicksburg, Miss. Miscellaneous paper ; A-79-6.
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